89* Moli1901 (duramycin) increases chloride transport in cystic fibrosis airway epithelial and pancreatic cell lines

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The lantibiotic Moli1901 is a polycyclic peptide derived from *Streptomyces cinnamoneum*, which has been reported to stimulate chloride (Cl^-) efflux from airway epithelial cells, and therefore it has been suggested as a potential drug for the treatment of cystic fibrosis (CF).

In the present study, we investigated the effect of Moli1901 on Cl^- efflux from normal human bronchial epithelial (16HBE) cells, a CF airway epithelial (CFBE) cell line, a normal pancreatic epithelial cell line (PANC-1), a CF pancreatic cell line (CFPAC-1), normal human airway submucosal gland cells (Calu-3), CF airway submucosal gland cells (CFSME), and untransfected baby hamster kidney fibroblasts (BHK-wt).

Cl^- efflux was determined with the help of a fluorescent probe, N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE).

It was found that Moli1901 dissolved in Standard Ringer's solution without Cl^- ions (SR0) significantly stimulated Cl^- efflux from CFBE cells (at a concentration of 1μM), from CFPAC cells (at 3μM) and from Calu-3 cells (at 1 and 10 μM). In these cells, stimulated Cl^- efflux was significantly increased over basal Cl^- efflux, and a stimulation corresponding to 30–40% of wild-type CFTR activity was obtained. Further experiments in the presence of inhibitors of CFTR (inhibitor-172), of Ca^{2+}-activated Cl^- channels (gadolinium), and of the calcium ionophore A23187 were performed.

These results show that Moli1901 causes a CFTR-independent Cl^- efflux, which may explain the beneficial action of Moli1901 on FEV_1, recently documented in a short-term clinical trial (Chest 131:4161; 2007).

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90* Safety, tolerability and efficacy of multiple doses of aerosolised Moli1901 in adolescents and adults with cystic fibrosis

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Moli1901 activates a Ca^{2+}-dependent alternative Cl^- channel with the potential to compensate for CFTR deficiency.

Two phase II randomised, placebo-controlled, double-blinded studies were performed in CF patients with stable lung disease and a FEV_1 >60% predicted. A multiple (5 days), rising-dose (0.5, 1.5, or 2.5 mg/d of Moli1901) study was conducted in 24 patients ⩾16 yrs (I) (Grasemann H et al., Chest 2007). In a second study, 2.5 mg/d Moli1901 or placebo were administered once daily for 28 days in 18 patients (9 ⩾16, and 9 ⩾12 yrs) (II). Over periods of 4 (I) and 8 (II) wks, adverse events (AEs), spirometry, pulse oximetry and quality of life were assessed.

In I, Moli1901 was well tolerated in all but 2 patients (1: transient significant decrease in FEV_1 following inhalation which resolved spontaneously, treatment discontinued; 2: transient throat numbness during inhalation). In II, no significant AE was observed; the most frequent AEs were cough and dry throat, most of them resolved within 1 hour after inhalation. Both trials were not designed to show efficacy; however, a significant difference in the median change in FEV_1 from day 1 to day 5 was observed between the 2.5 mg/d Moli1901 and the placebo group in I ($p = 0.01$), and in the median change in FEV_1 from day 1 to day 56 between the Moli1901 and placebo group in II ($p = 0.02$).

Moli was well tolerated in both trials, and appears to be safe in adolescent and adult CF patients. In addition, Moli1901 had a beneficial effect on pulmonary function, which supports further investigation of its efficacy.

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91* Preventive but not late amiloride therapy reduces morbidity and mortality of cystic fibrosis-like lung disease in mice

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Increased airway Na^+ absorption is a characteristic abnormality in the pathogenesis of cystic fibrosis (CF) lung disease. We previously demonstrated that mimicking accelerated Na^+ transport in mice by airway-specific overexpression of epithelial Na^+ channels (ENaC) caused dehydration of airway surfaces, deficient mucus clearance, and a spontaneous lung disease sharing key features with CF in humans. In the present study, we used βENaC-transgenic (βENaC-Tg) mice to test if inhibition of increased Na^+ absorption by the ENaC blocker amiloride has therapeutic effects on CF-like lung disease *in vivo*. βENaC-Tg and wild-type mice were treated by intranasal instillation of amiloride or vehicle for a period of 14 days. Subsequently, mice were killed, bronchoalveolar lavage (BAL) performed, and lungs processed for histology. We show that early amiloride treatment, i.e., from the first day of life, significantly reduced pulmonary mortality, airway mucus obstruction, and airway inflammation in βENaC-Tg mice. In contrast, consistent with previous human trials in CF patients, amiloride did not have benefits if treatment was started after the development of CF-like lung disease in βENaC-Tg mice.

We conclude that preventive inhibition of increased airway Na^+ absorption provides an effective therapy for CF-like lung disease *in vivo*. These results suggest that ENaC blocker treatment may be an effective preventive therapy for patients with CF if initiated early in life prior to the onset of lung disease.

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92 Duration of decreasing the nasal potential difference by amiloride–mannitol dry powder

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Hyperabsorption of sodium and subsequent water loss are believed to account for high sputum viscoelasticity in cystic fibrosis (CF). Problems were the time consuming inhalation of amiloride solution and a limited duration of the sodium blocking effect. Therefore the duration of an innovative amiloride/mannitol dry powder formulation administered in the nasal cavity was examined with nasal potential difference measurement (nPD).

In 7 CF patients (15 to 42 years) and 15 healthy persons (21 to 36 years) the acting of nPD-changing before and after nasal administration of amiloride/mannitol dry powder was examined. Basal nPD was measured subcutaneously in CF patients and controls. Then amiloride/mannitol dry powder was administered (composition: 1 mg amiloride//24 mg mannitol) nasally via an Aerolizer. 5 minutes after insufflation the nPD was recorded again and the duration of nPD-decrease was determined. The half-time-value was defined by the time until the nPD reached 50% of basal nPD.

The study was approved by the ethical committee of the University of Giessen.

In 7 CF patients the nPD decreased about 74±11%. The max. nPD decrease was reached after 27±19 minutes. The time until the nPD reached 50% of basal nPD was reached after 137±52 minutes.

In 15 healthy controls the max. nPD-decrease amounted 75±14% and was reached after 44±31 minutes. The time until nPD reached 50% of basal nPD was 106±44 minutes.

Amiloride/mannitol dry powder is effective and decreases the nasal potential difference until 75% of basal nPD in CF patients and healthy controls. The duration of decreasing is between 1.5 to 2 hours. The new innovative amiloride/mannitol dry powder formulation seems to be more practicable and saves time.